JPPIPA 11(12) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Formulation and Nutrient Component Analysis of Various Cost-Effective Bacterial Culture Media Based on Horticultural Waste

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Received: October 06 2025
Revised: November 22, 2025
Accepted: December 25, 2025
Published: December 31, 2025

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DOI: 10.29303/jppipa.v11i12.13545

Abstract: Horticultural waste, which is often overlooked, has great potential as an alternative raw material for cost-effective and environmentally friendly culture media. This study aims to develop a cost-effective alternative bacterial culture media formulation by utilizing horticultural waste. In addition, this study also aims to analyze the nutritional components of the formulated media to ensure its effectiveness in supporting bacterial growth. The results showed that *E. coli* was able to grow on all types of test media. This indicates slower growth compared to the control media. The average number of *E. coli* colonies on BW Agar and CW Agar at concentrations of 10%, 15%, and 20% after incubation for 48 hours was >300 CFU/mL. Meanwhile, the average number of E. coli colonies on PW Agar at concentrations of 10%, 15%, and 20% was 126 × 10^7 CFU/mL, 171 × 10^7 CFU/mL, and 225 × 10^7 CFU/mL, respectively. The number of S. aureus colonies was only obtained on BW Agar with concentrations of 10%, 15%, and 20%, namely 77 × 10^7 CFU/mL on BW Agar 10%, 200 × 10^7 CFU/mL on BW Agar 15%, and 300 × 10^7 CFU/mL on BW Agar 20%. Based on these results, the optimal formulations representing each type of test media selected for nutrient content analysis were BW Agar 20%, CW Agar 20%, and PW Agar 20%.

Keywords: Alternative bacterial media; Formulation; Horticultural waste; Macronutrient analysis; Micronutrient analysis.

Introduction

Culture media are essential components in microbiological studies and are used for various analytical processes to investigate microorganisms, including bacteria. Careful selection and preparation of culture media are required to ensure optimal microbial growth (Bonnet et al., 2020; Orekan et al., 2021). In many research and educational settings, commercial culture media remain the primary choice. However, their high cost can be a limiting factor, particularly in developing countries such as Indonesia (Poba et al., 2019). For example, Nutrient Agar one of the most widely used commercial media currently costs approximately IDR 850,000 to IDR 2,500,000 per 500 g. This condition has encouraged researchers to explore alternative media derived from readily available and low-cost materials.

As an agrarian country, Indonesia has an abundance of food crops with high nutritional value, including horticultural products. Fruits and vegetables, as major horticultural commodities, contain diverse complex and simple nutrients suitable for human consumption. Consequently, the demand for fruits and vegetables in Indonesia continues to increase in line with population growth and shifting dietary patterns (Dzulqaidah et al., 2021). This trend results in the rising production of organic waste, such as fruit and vegetable peels. Moreover, organic waste management in Indonesia remains suboptimal.

How to Cite:
Handarini, Sopiyani, P., Farizal, D., MK, A. S. A. F., & Fadhilah, W. K. (2025). Formulation and Nutrient Component Analysis of Various Cost-Effective Bacterial Culture Media Based on Horticultural Waste. *Jurnal Penelitian Pendidikan IPA*, *11*(12), 1098-1105. https://doi.org/10.29303/jppipa.v11i12.13545

Organic waste often accumulates at temporary or final disposal sites, causing environmental problems such as unpleasant odors, hazardous leachate, and methane production which may pose an explosion risk if accumulated over long periods (Santos et al., 2021). On the other hand, horticultural waste contains nutrients that can be utilized (Lobo & Dorta, 2019). The nutrient compositions of various fruit and vegetable peels have been documented (Sadef et al., 2022). For instance, 100 g of banana peel contains carbohydrates (18.5 g), protein (0.32 g), fat (2.11 g), minerals (calcium 715 mg, phosphorus 117 mg, iron 1.6 mg), and vitamins (B 0.12 mg, C 17.5 mg) (Ciccoritti et al., 2024).

Pineapple peel is also rich in macronutrients (carbohydrates 55.5 g, protein 5.11 g, fat 5.31 g) and minerals (calcium 8.3 mg, zinc 6.46 mg, iron 25.5 mg, manganese 5.32 mg) (Kumari & Tiwari, 2023). Among vegetables, 100 g of carrot peel contains substantial carbohydrates (6.03%) and protein (1.67%) but low fat (0.15%) (Ansari et al., 2023). Additionally, carrot peel is abundant in minerals and vitamins, including calcium (121 mg), sodium (206 mg), magnesium (167 mg), iron (29 mg), manganese (11 mg), zinc (11 mg), potassium (1123 mg), and phosphorus (232 mg) (Romelle et al., 2018). These data indicate that the nutrient profiles of horticultural waste have the potential to support bacterial growth. In general, bacteria require carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, calcium, zinc, potassium, copper, manganese, magnesium, iron, vitamins, water, and energy (Bhalta et al., 2023).

Several researchers in Indonesia have reported the potential use of alternative media for bacterial and fungal growth derived from various local food crops. However, most studies have focused on utilizing tubers (Hermsen et al., 2015; Wang, 2019), legumes (Alteri et al., 2019; Alteri & Mobley, 2015; Hermsen et al., 2015; Jiao et al., 2024; Poba et al., 2019), and seeds (Ansari et al., 2023; Biase & Lund, 2015; Dzulqaidah et al., 2021; Li et al., 2024), which are still widely consumed as food sources. Other studies by (Handarini et al., 2018) have evaluated rice-washing water agar as a culture medium for *Aspergillus niger, Candida albicans*, and *Penicillium* sp. (Elsas et al., 2011; Machado et al., 2019)). Therefore, research on the formulation and nutritional analysis of bacterial culture media derived from horticultural waste is highly promising, offering a potential solution to both the high cost of commercial media and the challenges associated with organic waste management in Indonesia.

Method

The study employed a descriptive research design. The research workflow comprised five sequential stages: (1) preparation; (2) testing of multiple formulations of three culture media types for bacterial growth; (3) analysis of the macro- and micronutrient composition of the three alternative culture media; and (4) an initial evaluation of production costs.

Identification of Escherichia coli and Staphylococcus aureus

The bacterial strains used in this study were pure cultures obtained from the Microbiology Laboratory, Faculty of Health Sciences, Ikes Rajawali. Prior to use, each pure culture was re-identified to confirm species accuracy. Identification was performed using the VITEK-MS system.

Collection and Processing of Banana, Pineapple, and Carrot Peel Waste

Banana, pineapple, and carrot peel waste was collected from various food vendors who processed these three commodities. Efforts were made to ensure that the peel waste originated from the same varieties of bananas, pineapples, and carrots. Each type of peel waste was collected and subsequently dried under sunlight until a constant dry mass was obtained. The dried material was then ground into powder form. The resulting powders were stored in tightly sealed containers.

Preparation of Culture Media and Bacterial Growth Testing on Three Alternative Culture Media

The alternative culture media were prepared by first formulating the compositions based on previous studies. Powdered banana peel, pineapple peel, and carrot peel were weighed in three variations: 10 g, 15 g, and 20 g. Each powder sample was placed into an Erlenmeyer flask, supplemented with 2 g of solidifying agar, and dissolved in 100 mL of distilled water. The pH of the media was measured and adjusted to a range of 6.5–7.0. The media were sterilized in an autoclave at 121 °C and 2 atm for 15 minutes.

Testing of the Three Culture Media Formulations

The performance of each formulation was evaluated by inoculating 1 µL of bacterial inoculum (cell density 10^6 CFU/mL) of *E. coli* and *S. aureus* onto the test media. Inoculation was carried out using the spread plate technique. After inoculation, the media were incubated at 37 °C for 18–24 hours. Colony growth was observed and counted on the following day.

Macro- and Micronutrient Analysis of the Three Alternative Culture Media

Macro- and micronutrient analyses were performed for each type of alternative culture medium, specifically on the formulations that demonstrated the most optimal bacterial growth. Macronutrient analysis included total

protein using the Kjeldahl method, total fat using the Soxhlet method, and total reducing sugars using the Luff-Schoorl method. Micronutrient analysis included mineral content (Ca, Fe, K, Mg, Mn, Na) using AAS, and vitamin B1, B2, and B6 levels using HPLC.

Initial Evaluation of Production Costs

The initial cost evaluation was conducted by calculating all material costs (price per kilogram of peel waste, solidifying agar, and distilled water), equipment usage costs, and the cost of nutrient composition analysis.

Result and Discussion

The identification results confirmed that the pure cultures used for testing were correctly identified as *Escherichia coli* and *Staphylococcus aureus*. Both bacterial species were subsequently used in the preliminary performance testing of BW Agar, CW Agar, and PW Agar, each prepared in three concentration levels. Observations from the preliminary test after 24 hours of incubation showed no growth of either *E. coli* or *S. aureus*. Therefore, incubation was extended to 48 hours under the assumption that the bacteria might be undergoing a lag (adaptation) phase or exhibiting a slower growth rate compared to the control medium (nutrient agar). However, observations after 48 hours of incubation also showed no bacterial growth in any of the media variations. Based on these results, nutritional optimization of the test media was deemed necessary to support more optimal bacterial growth. In this study, optimization was conducted by adding 2 g of dextrose monohydrate as a carbon source and 0.3 g of beef powder as a nitrogen source. The performance test results for the optimized media are presented in the Table 1.

Tabel 1. Performance Evaluation Results of Banana Peel (BW Agar), Carrot Peel (CW Agar), and Pineapple Peel (PW Agar) Media

Media	Number of cell *E. coli* ($x\ 10^7$ CFU/mL)	Note
Nutrient Agar (kontrol)	>300	grown after 24 hours of incubation
BW Agar 10 %	>300	grown after 48 hours of incubation
BW Agar 15 %	>300	grown after 48 hours of incubation
BW Agar 20 %	>300	grown after 48 hours of incubation
CW Agar 10 %	>300	grown after 48 hours of incubation
CW Agar 15 %	>300	grown after 48 hours of incubation
CW Agar 20 %	>300	grown after 48 hours of incubation
PW Agar 10 %	126	grown after 48 hours of incubation
PW Agar 15 %	171	grown after 48 hours of incubation
PW Agar 20 %	225	grown after 48 hours of incubation

Data presented in Table 2 indicate that *E. coli* colonies began to show growth on all types of test media after 48 hours of incubation. This reflects slower growth compared to the control medium. In contrast, *S. aureus* exhibited growth only on BW Agar after 48 hours of incubation. Several factors may contribute to this delayed growth, including an imbalance in nutrient composition. Nutrient Agar (NA), as a standard medium, is specially formulated with adequate sources of carbon (peptone), nitrogen, minerals, and vitamins to support optimal microbial growth (Udofa et al., 2002). Meanwhile, banana, carrot, and pineapple peels likely contain more limited nutrients—such as higher fiber or simple sugars but lower protein/peptide content as nitrogen sources (Ciccoritti et al., 2024; Kumari & Tiwari, 2023).

It is also presumed that the bacteria required additional time to adjust their metabolism to utilize nutrients derived from plant-based substrates, which are more complex than the readily available nutrients in NA. Banana peel contains simple carbohydrates (glucose, fructose, sucrose), low protein and nitrogen levels, starch, fiber (cellulose, hemicellulose, lignin), and minerals such as K, Mg, and Ca (Ansari et al., 2023; Kumari & Tiwari, 2023; Romelle et al., 2018). The presence of simple sugars may adequately support the growth of *E. coli* and *S. aureus*; however, limited nitrogen availability and high fiber content likely contribute to an extended lag (adaptation) phase for both bacteria. During the lag phase, cells do not divide rapidly while undergoing physiological adjustments (Bertranda, 2019). This adaptation includes synthesizing enzymes required to degrade complex substrates such as starch and fiber, and modifying membrane transport mechanisms to absorb available nutrients. Additionally, bacteria may exhibit diauxic growth, prioritizing simple sugars (e.g., glucose) before shifting to more complex substrates, resulting in a temporary growth pause (Hermsen et al., 2015; Wang, 2019). On nutrient-rich media such as NA, the lag phase is short; on plant-based media, the lag phase is prolonged, producing visibly slower growth.

Tabel 2. Performance Evaluation Results of Banana Peel (BW Agar), Carrot Peel (CW Agar), and Pineapple Peel (PW Agar) Media

Media	Number of cell *S. aureus* ($x\ 10^7$ CFU/mL)	Note
Nutrient Agar (kontrol)	>300	grown after 24 hours of incubation
BW Agar 10 %	77	grown after 48 hours of incubation
BW Agar 15 %	200	grown after 48 hours of incubation
BW Agar 20 %	300	grown after 48 hours of incubation
CW Agar 10 %	0	not grown
CW Agar 15 %	0	not grown
CW Agar 20 %	0	not grown
PW Agar 10 %	0	not grown
PW Agar 15 %	0	not grown
PW Agar 20 %	0	not grown

Overall, the performance test results demonstrated that both bacteria were able to grow on all media types, with the lowest *E. coli* colony count observed on 10% PW Agar. In contrast, *S. aureus* grew only on BW Agar. This indicates that BW Agar has the greatest potential among the tested horticultural-waste–based media to support bacterial growth. Banana peel is known to contain carbohydrates capable of supporting microbial proliferation (Ansari et al., 2023). The difference observed in *S. aureus* performance is likely influenced by the distinct physiological characteristics of each bacterium.

Escherichia coli possesses a more flexible metabolic capability, enabling it to utilize a wide variety of carbon and nitrogen sources. It can metabolize non-glucose carbon substrates through regulatory mechanisms. Carrot and pineapple peels contain simple sugars, fiber, and other organic compounds that *E. coli* can exploit (Alteri et al., 2019; Alteri & Mobley, 2015). Systems biology studies highlight the metabolic versatility of *E. coli* under diverse nutrient conditions (Jiao et al., 2024). Furthermore, *E. coli* is relatively tolerant to variations in pH and to phenolic or organic acid compounds often found in plant tissues (Biase & Lund, 2015; Elsas et al., 2011; Li et al., 2024).

In contrast, the Gram-positive *S. aureus* is presumed to require specific nitrogen sources, vitamins, and amino acids that may not be sufficiently available in carrot and pineapple peel waste. Banana peel medium is more suitable for *S. aureus* due to its relatively high simple sugar content (glucose, fructose, sucrose) and near-neutral pH. Although *S. aureus* is heterotrophic, it has more stringent nutritional requirements (Machado et al., 2019). It can utilize simple sugars but is less efficient at degrading complex carbohydrates or plant polysaccharides. *S. aureus* requires essential amino acids (e.g., proline, arginine, valine) and certain vitamins (e.g., niacin, thiamine), which may be lacking in sufficient quantities in plant-based substrates (Reslane et al., 2024). Without these specific nutrients, growth is rapidly inhibited.

Moreover, *S. aureus* prefers neutral to slightly alkaline pH (6.5–7.5). Media prepared from pineapple peel (PW Agar)—naturally acidic due to organic acids—may inhibit its growth and metabolism. Pineapple peel contains high levels of organic acids such as citric, malic, ascorbic, oxalic, tartaric, ferulic, and p-coumaric acids, some of which are phenolic compounds with antimicrobial properties (Andriani et al., 2013; Astrinia, 2022; Egeten et al., 2016). Carrot peel contains carotenoids and phenolic compounds that may exert antimicrobial activity against Gram-positive bacteria such as *S. aureus* (Ahmad et al., 2019).

Further results from the performance test (Table 1) show that BW and CW Agar supported *E. coli* growth more effectively than PW Agar. At the 10% concentration, *E. coli* colony counts were already comparable to the control (>300), although growth occurred significantly later. This suggests that BW and CW Agar contain nutrient components more suitable for *E. coli*. Nutritional composition data indicate that 100 g of banana peel contains 18.5 g carbohydrate, 0.32 g protein, 2.11 g fat, and minerals such as calcium (715 mg), phosphorus (117 mg), and iron (1.6 mg), along with vitamins B (0.12 mg) and C (17.5 mg) (Ansari et al., 2023). One hundred grams of carrot peel contains high carbohydrates (6.03%), protein (1.67%), and low fat (0.15%) (Tame & Hanson, 2024). These nutritional contents will be further analyzed according to media formulation in subsequent stages of the study. Besides nutrient content, the acidity levels of banana peel (pH ~5.0–5.5) and carrot peel (pH ~6.0) are closer to neutral compared to pineapple peel (pH ~3.5–4.0). *E. coli* grows optimally at pH 6.0–7.0; therefore, banana and carrot peel media are more favorable. Pineapple peel contains bromelain and high concentrations of antimicrobial organic acids (citric, malic, ascorbic), which suppress *E. coli*. Banana and carrot peels also contain bioactive compounds, but at lower concentrations than pineapple peel, and thus exert less inhibitory effect.

These performance test results were subsequently used to determine the most optimal media formulations for supporting the growth of both bacteria. The optimal

formulation was defined as the medium with the lowest concentration that produced colony counts closest to the control. Based on this criterion, the optimal formulations for each medium type were BW Agar 20%, CW Agar 20%, and PW Agar 20%. These three formulations were then subjected to macro- and micronutrient analysis.

Macro- (total sugars, total protein, total fat) and micronutrient (Mg, K, Fe, Ca, Na, vitamin B1, B2, and B6) analyses were conducted on the three formulations considered most effective in supporting the growth of *E. coli* and *S. aureus*. This analysis was intended to confirm that media derived from banana, carrot, and pineapple peels are capable of supplying sufficient energy sources, nitrogen, minerals, and enzymatic cofactors required for bacterial growth. Such analysis is essential to identify which nutrients serve as limiting factors, to elucidate differences in media performance, and to determine whether the nutrient composition of low-cost formulations is adequate compared to standard media. The complete macro- and micronutrient analysis results are presented in Table 3.

Tabel 3. Macronutrient and Micronutrient Analysis Results of BW Agar 20%, CW Agar 20%, and PW Agar 20% Media

Parameter	BW Agar 20%	CW Agar 20%	PW Agar 20%	Testing Method
Gula Total	3.16 %	2.62 %	1.39 %	Luff Schoorl
Protein Total	0.49 %	0.34 %	0.21 %	Kjeldahl
Lemak Total	0.19 %	0.58 %	0.16 %	Soxhlet
Kalsium (Ca)	241.63 mg/kg	271.90 mg/kg	269.39 mg/kg	
Magnesium (Mg)	63.55 mg/kg	54.01 mg/kg	56.16 mg/kg	AAS
Besi (Fe)	15.91 mg/kg	14.48 mg/kg	11.10 mg/kg	
Kalium (K)	331.04 mg/100g	316.27 mg/100g	115.09 mg/100g	ICP-OES
Natrium (Na)	94.92 mg/100g	153.26 mg/100g	237.58 mg/100g	
Vitamin B1(Tiamin)	not detected	not detected	not detected	
Vitamin B2 (Riboflavin)	not detected	not detected	0.60	UPLC-PDA
Vitamin B6 (Piridoksin)	not detected	0.15 mg/100g	not detected	

The analysis presented in Table 3 shows clear variations in the macronutrient and micronutrient composition of each media formulation. These variations contribute to the differences observed in the growth performance of *E. coli* and *S. aureus* across the tested media. In terms of macronutrients, the results indicate that BW Agar contains the highest total sugar content (3.16%), followed by CW Agar (2.62%), with the lowest level found in PW Agar (1.39%). Sugar content serves as a crucial carbon and energy source, directly influencing the acceleration of bacterial transition into the exponential growth phase. Recent studies demonstrate that increased carbon availability enhances *E. coli* growth by strengthening glycolytic flux and enabling efficient metabolic regulation (You et al., 2013). Accordingly, BW Agar offers stronger growth support compared to CW Agar and PW Agar.

The total protein content further shows that BW Agar has the highest value (0.49%), followed by CW Agar (0.34%) and PW Agar (0.21%). Protein functions as an important nitrogen source for enzyme synthesis and cellular structural components. Nitrogen availability strongly influences the ability of bacteria to increase DNA replication rate and biomass production (Gorochowski et al., 2016; Schmidt et al., 2016) . The high protein content in BW Agar makes it suitable for the stable growth of both bacteria, particularly *S. aureus*, which requires elevated nitrogen levels for forming the thick peptidoglycan layer characteristic of Gram-positive bacteria. This is consistent with performance test data showing that CW Agar and PW Agar do not support *S. aureus* growth. Protein levels of 0.34% and 0.21% are insufficient to meet the nutritional demands of *S. aureus*. Conversely, differences in total lipid content among the media are minimal and physiologically have negligible impact on the metabolism of heterotrophic bacteria such as *E. coli* and *S. aureus*. Modern studies confirm that lipids are not major energy sources for these organisms, although they contribute slightly to membrane integrity (Parsons & Rock, 2013). Thus, lipid content does not constitute a key factor underlying differences in bacterial growth.

In terms of micronutrients, BW Agar contains the highest concentrations of Mg (63.55 mg/kg) and Fe (15.91 mg/kg). Several studies highlight the importance of Mg in ribosomal stability, polymerase activity, and translation processes, particularly in Gram-negative bacteria (Bédard et al., 2024). Iron is also a key component of respiratory enzymes and ATP generation. Research further emphasizes that Fe availability affects the aerobic growth rate of opportunistic bacteria (Nairz et al., 2010). These mineral advantages explain why BW Agar exhibits performance most comparable to nutrient agar.

CW Agar, on the other hand, contains the highest calcium concentration (271.90 mg/kg). Calcium plays a

role in enzyme stability, membrane stress response, and cell wall fortification, especially in Gram-positive bacteria such as *S. aureus* (Domínguez, 2018). The high calcium content in CW Agar could potentially support the growth of this bacterium. However, performance test results showed that *S. aureus* failed to grow on CW Agar. This indicates that calcium alone is not a determining factor for successful bacterial growth. Other factors appear to exert more dominant effects on the viability of *S. aureus* on this medium, particularly macronutrient limitations such as insufficient carbon and nitrogen sources. *Staphylococcus aureus* requires relatively high nitrogen availability to support synthesis of its thick peptidoglycan layer; thus, nitrogen deficiency inhibits biomass formation and disrupts cell division. In addition, sugar content in CW Agar (2.62%) is insufficient to provide the energy required for entering the exponential phase. Limited carbon availability can prevent cells from exiting the lag phase, ultimately resulting in no colony formation (You et al., 2013).

Another contributing factor is nutrient imbalance. Optimal bacterial growth requires a balanced ratio of carbon, nitrogen, minerals, and growth factors. When one nutrient is excessive while others are deficient, biosynthetic processes become disrupted, compromising cellular resource allocation. Recent studies show that nutrient imbalance can reduce growth rates even when some nutrients are supplied in abundance (Gorochowski et al., 2016; Schmidt et al., 2016). Although calcium is high in CW Agar, the low availability of carbon and nitrogen and the potential presence of inhibitory compounds collectively hinder *S. aureus* growth.

In contrast, micronutrient analysis of PW Agar revealed the lowest potassium (115.09 mg/100 g) and the highest sodium content (237.58 mg/100 g). Potassium is essential for bacterial cellular homeostasis, osmotic regulation, and activation of intracellular enzymes. Potassium deficiency can decrease viability and prolong the lag phase in non-halophilic bacteria. Conversely, high sodium levels may create hypertonic conditions that inhibit *E. coli* growth, as reported in recent bacterial osmoregulation studies (Wood, 2015). This combination of low potassium and high sodium explains why PW Agar generally supports slower growth than BW and CW Agar. Vitamin B levels across all media were extremely low, with only PW containing B2 and CW containing small amounts of B6. However, modern studies indicate that bacteria such as *E. coli* can synthesize most B vitamins endogenously when sufficient carbon and nitrogen sources are available (Price et al., 2018). Therefore, vitamin levels are not considered major contributors to performance differences among the media.

Overall, BW and CW Agar exhibit a more balanced nutrient composition compared to PW Agar, enabling growth performance more closely resembling that of nutrient agar. BW is superior in providing energy sources and essential minerals, whereas CW is superior in calcium content. PW Agar, meanwhile, experiences multiple nutrient limitations that lead to the slowest growth. These findings support the potential use of horticultural waste as an alternative source of bacterial culture media, provided that nutrient composition is adequate.

Conclusion

The types of alternative media and optimal formulations capable of supporting the growth of *E. coli* and *S. aureus* were identified as banana peel waste media (BW Agar) at a concentration of 20%. Meanwhile, CW Agar 20% and PW Agar 20% were found to be optimal only for supporting the growth of *E. coli*.

Acknowledgments

We would like to express our gratitude to the Ministry of Higher Education, Science, and Technology for providing research funding through the Beginner Lecturer Research Grant scheme (Penelitian Dosen Pemula) in 2025.

Author Contributions

Conceptualization, H.; methodology, H., and P.S.; resources, H., P.S., D.F., A.S.A.F.MK., and W.K.F.; writing of the original draft, preparation of the manuscript by H., P.S., D.F., A.S.A.F.MK., and W.K.F.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

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